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14. ABSTRACT
Many algae including Chlamydomonas accumulate triacylglycerols when cultures enter stationary phase leading to nutrient limitation. The identification of microalgal genes encoding the enzymes and regulatory factors required for the induction of oil biosynthesis is the immediate goal of the proposed work. Towards this end the following findings were met:
1. A mutant screen to isolate gene disruption mutants of Chlamydomonas was established and 15 mutants were identified with altered lipid composition of a pool of 7000 tested transgenic lines.
2. Conditions were established and gene expression profiles were compared under high and low N growth conditions. 700 genes were found to be differentially regulated, among these 5 encoding transcription factors.
3. Oil bodies were isolated from induced Chlamydomonas and approximately 250 proteins associate with these oil bodies were identified.
4. Four predicted enzymes of oil biosynthesis were isolated and cloned into yeast expression vectors.
These genes and mutants are currently under investigation for their potential roles in oil biosynthesis in microalgae.

15. SUBJECT TERMS
Target genes for oil engineering, transcriptional profile comparison, lipid mutants, microalgae oil biosynthesis, enzymes involved in oil body formation in micro algae

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Final Report of Pilot Project Phase 03/15/07 – 04/30/08 Entitled:

Regulation of oil biosynthesis in algae

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Summary
The widely recognized need for the development of biomass-based domestic production of high energy liquid transportation fuels can potentially be addressed by exploring oil (triacylglycerol) biosynthesis in microalgae. Many algae including Chlamydomonas accumulate triacylglycerols when cultures encounter certain environmental stresses such as nutrient limitation. However, the regulatory factors and enzymes that govern triacylglycerol biosynthesis in algae have not been studied in detail at the molecular level. Toward this end, we have initiated a study in the model green alga Chlamydomonas reinhardtii with both global and focused approaches aimed at understanding regulation of TAG biosynthesis in algae. Genetic mutant screens have been developed for loss of triacylglycerol accumulation under induced conditions, and for gain of triacylglycerol biosynthesis under non-induced conditions. Mutants have been identified and their biochemical and physiological characterization has begun. A high-throughput cDNA pyrosequencing experiment has been conducted under induced and non-induced conditions to generate a deep set of expressed sequence tags for comparative transcriptional profiling. In addition, the triacylglycerol storage compartment of the cell has been purified and the associated proteins have been identified by MS/MS approaches. A more targeted effort was focused on candidate genes encoding diacylglycerol acyltransferases the predicted key enzymes of triacylglycerol biosynthesis. The newly identified genes and the functional genomic information will provide novel targets for future engineering approaches towards optimizing microalgae oil production strains.

Major Accomplishments and Findings:
The following findings were made during the report period:

1. Plasmid disruption mutagenesis of Chlamydomonas was conducted. 15 putative Mutants producing oil under non-inducing conditions (gain-of-function mutants) as well as oil-deficient mutants under inducing conditions (loss-of-function mutants) were identified by Nile Red staining followed by direct lipid analysis of cell extracts. Nitrogen starvation conditions leading to oil accumulation have been optimized. PCR-based approaches will allow the identification of mutant loci shortly.
2. The genome-wide abundance of mRNAs was determined using multiparallel pyrosequencing of cDNAs derived from cells grown under inducing and non-inducing conditions. The abundance of gene-specific tags provides a measure of gene expression and will aide in the identification of gene sets (regulons) coordinately expressed during oil biosynthesis. ~93 Mbp were sequenced (~500,000 ESTs); ~6000 genes with one or more hit were identified. ~700 genes were differentially expressed. Among these were 5

transcription factors that represent particularly interesting targets for future engineering purposes

*3. **Biochemical analysis of diacylglycerol acyltransferases (DGATs)**.* These are key enzymes of oil biosynthesis catalyzing the assembly of triacylglycerol in many organisms. 5 Genes predicted to encode DGATs and their role in triacylglycerol biosynthesis were identified, cloned into yeast expression vectors and introduced into a yeast mutant unable to produce triacylglycerol. The resulting yeast strains will be available for the production and biochemical characterization of the recombinant proteins.

*4. **Isolation and characterization of oil bodies**.* Oil bodies storing the triacylglycerol during inducing growth conditions were isolated and separated by differential centrifugation from other cell organelles. The oil bodies were extracted and proteins were analyzed by gel electrophoresis. Sections of the gel were subjected to in-gel digestion with proteases. The protein fragments were identified by mass spectrometry. Approximately 250 proteins were identified. Of these 17 were predicted to be involved in lipid metabolism. These are targets for further study and possibly engineering of oil body formation or maintenance.

Deviation from Original Proposal

Proteomics of oil bodies was not originally proposed. However, because oil bodies could be easily isolated from Chlamydomonas, the opportunity to characterize this organelle was pursued. Querying the mRNA profiling data and the proteomics data provides a powerful tool to narrow down genes relevant for oil body formation or oil accumulation in microalgae.

Publications of Findings

Due to the short duration of this pilot grant, no publications have been prepared. However, as follow up studies are ongoing, it is expected that these finding will become part of publications currently under preparation.

Outreach and Education

The project was conducted by two graduate students (E. Moellering and R. Miller) and one undergraduate student (M. Fedewa). R. Miller conducted the profiling study and the cloned the genes encoding biosynthetic enzymes, E. Moellering and M. Fedewa, worked on the mutant isolation, and E. Moellering did the oil body proteomics study.

Concluding Remarks

The reported findings of this pilot study need to be considered preliminary. A follow up study is in progress which builds on the reported findings. Publications are currently under preparation that will publically report the detailed findings in peer reviewed Journals.